Synthesis of Phenstatin and Prodrugs Thereof

The present invention relates generally to compounds for use in the treatment of one or more neoplastic diseases and more particularly to the synthesis of the antineoplastic compound herein denominated "phenstatin" and water soluble prodrugs thereof.

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By way of background, the elucidation and isolation of combretastatin A-4 is described in U.S. Patent No. 4,996,237 which issued to G.R. Pettit et al., on February 26, 1991, while early efforts to develop a combretastatin A-4 prodrug are described in U.S. Patent No. 5,561,122, which issued to G.R. Pettit on October 1, 1996. The general background information from each of those patents is incorporated herein by this reference thereto.

The potent cancer cell growth and tubulin assembly inhibitor combretastatin A-4 was originally isolated from the African tree *Combretum caffrum* (*Combretaceae*) circa 1985 and has been undergoing clinical development. The present disclosure comprises a benchmark in the continuing effort to synthesize practical water soluble prodrugs related to combretastatin A-4 and is a significant and remarkably unexpected extension of those early efforts which are described in U.S. Patent No. 5,561,122, *supra*.

The African willow tree *Combretum caffrum* Kuntze (*Combretaceae*) has proven to be a very productive source of cancer cell growth (murine P388 lymphocytic leukemia) inhibitory stilbenes, bibenzyls and phenanthrenes (See: Pettit, et al., Antineoplastic Agents 291. Isolation and Synthesis of Combretastatins A-4, A-5, and A-6, *J. Med. Chem.* 1995, *38*, 1666-1672). Since 1979 promising leads have been pursued which were focused on the three most active (inhibition of cancer cell growth and polymerization of tubulin (*Id.*))

constituents, namely combretastatin A-1 (**1a**), A-2 (**2**), and A-4 (**1b**) (*Id.*) (*See*, Figure 1). Of these, combretastatin A-4 (**1b**) has reached clinical development as the very soluble prodrug **1d**. Meanwhile other research groups have also been further extending structure/activity relationships (hereinafter referred to as "SAR") among the combretastatins (See: Bedford, et al., Synthesis of Water-Soluble Prodrugs of the Cytotoxic Agent Combretastatin A-4, *BioMed. Chem. Lett.* 1996, *6*, 157-160) and related stilbenes (See: Kitanaka, et al., Oligomeric Stilbenes from *Caragana chamlagu* LAMARK Root, *Chem. Pharm. Bull* 1996, *44*, 565-567).

Epoxidation of combretastatin A-4 silyl ether **(1c)** (Pettit et al., 1995, *supra*) using the Jacobsen chiral Mn (salen) complex (See: Popisil, et al., X-Ray Structural Studies of Highly Enantioselective Mn(salen) Epoxidation Catalysts, *Chem. Eur. J.* 1996, *2*, 974-980) resulted in rearrangement and oxidation products (indicated by ^{1}H-NMR and TLC analyses). Use of protecting groups for the phenol offered no improvement and the absence of a protecting group resulted in apparent polymerization.

In the case of combretastatin A-4, the formation of the oxirane by the Jacobsen oxidation was observed by ^{1}H-NMR, but the compound resisted isolation. In addition, the derived 1,1-diphenylacetaldehyde and benzophenone **3a** products appeared to be formed. Following isolation of silyl ether **3a** and mass spectral analysis of the suspected benzophenone (10% yield, **3b**) cleavage of its silyl ether protective group showed the loss of one carbon atom. A conjugated carbonyl peak at 1633 cm^{-1} was seen in the IR spectrum. Loss of the olefin bridge was established by NMR. This result suggested the need for structure confirmation of ketone **3b** by X-ray crystallography, which demonstrated the compound was 3-hydroxy-3',4,4',5'-tetramethoxybenzophenone (Figure 2). Finally, oxidative cleavage gave the silylated benzophenone **3a**.

Because of the potent cancer cell line growth inhibition displayed by the deprotected benzophenone, it was denominated "phenstatin." The structure

of phenstatin **3b** proved to be closely related to the aromatic system of podophyllotoxin **5**, a target of our efforts (Pettit, et al., Antineoplastic Agents V. The Aromatic System of Podophyllotoxin (Part B), *J. Med. Pharm. Chem.* 1962, *5*, 800-808), which began in 1958, to locate the structural core responsible for the antineoplastic activity of this important lignan.

In order to improve the availability of phenstatin by a more efficient synthesis, several alternative routes were explored. The general procedure reported (Pettit et al., 1962, *supra*) in 1962 for obtaining ketone **4a** based on a morpholine amide intermediate proved to be most effective. The phenstatin amide precursor was prepared from 3-(*tert*-butyldimethylsilyl)oxy-4-methoxybenzaldehyde by oxidation to the corresponding carboxylic acid using potassium permanganate, followed by conversion to the acid chloride, and finally treatment with morpholine (Scheme 2, Figure 4). The amide **6a** was allowed to react with the lithium derivative prepared from 3,4,5-trimethoxybromobenzene (See: Jung, et al., Stereoselective Synthesis of an Analogue of Podophyllotoxin by an Intramolecular Diels-Alder Reaction, *J. Org. Chem.* 1985, *50*, 1087-1105) and t-butyllithium at -78°C followed by deprotection to afford phenstatin **3b** in 30% overall yield. Similarly, a series of related **4** benzophenones were synthesized for SAR purposes as shown in Table 1 below.

Table 1. Physical Properties of the Amides and Benzophenones.

Compound	% Yield (from **5**)	cryst. solvent	MP (°C)	Formula (c)(d)
6a	94	hexane	66-67	$C_{18}H_{29}NO_4Si$
6b	88	(a)	87-88	$C_{13}H_{17}NO_2$
6c	73	(a)	78-79	$C_{13}H_{17}NO_4$
6d	78	(a)	84-85	
6e	97	(a)	78-79	$C_{13}H_{17}NO_4$
6f	100 (g)	(a)	117-119	$C_{11}H_{11}Cl_2NO_2$

Compound	% Yield (from **5**)	cryst. solvent	MP (°C)	Formula (c)(d)
6g	97 (g)	(b)	68-70	$C_{11}H_{11}F_2NO_2$
3a	76	hexane	74-75	$C_{23}H_{32}O_6Si$
3b	83	ethyl acetate-hexane	149-150	$C_{17}H_{18}O_6$
3c	72 (e)	n/a	clear oil at r.t.	$C_{31}H_{31}O_9P$
3d	(f)	water-acetone	165-167	$C_{17}H_{17}O_9Na_2P$
3e	93 (h)	ethyl acetate-hexane	164-165	$C_{19}H_{20}O_7$
4a	98	methanol	124-125	$C_{17}H_{16}O_6$
4b	75	hexane	104-105	$C_{18}H_{20}O_4$
4c	53	toluene-hexane	120-122	$C_{18}H_{20}O_6$
4d	70	hexane	121-122	$C_{18}H_{20}O_6$
4e	34	hexane	131-132	$C_{16}H_{14}Cl_2O_4$
4f	36	hexane	121-123	$C_{16}H_{14}F_2O_4$

Code: (a)=crystallized following concentration of the flash column chromatography solvent. (b)=crystallized at O°C upon isolation. (c)=All compounds were subjected to combustion analysis for C and H (and for Cl, F, and N where appropriate). The results were within $\pm$0.4% of the calculated values. (d)=The ^{1}H- and ^{13}C-NMR data at 300 MHZ was also consistent with the assigned structure. (e)=From **3b**. (f)=from **3c**. (g)=% Yield from acid chloride. (h)=% yield from phenstatin.

Because of the improved therapeutic effects of the combretastatin A-4 sodium phosphate **1d** prodrug (Pettit, et al., Antineoplastic Agents 322. Synthesis of Combretastatin A-4 Prodrugs, *Anticancer Drug Design* 1995, *10*, 299-309) when compared to the parent phenol **1b** (Pettit, et al., Isolation and Structure of the Strong Cell Growth and Tubulin Inhibitor Combretastatin A-4, *Experentia* 1989, *45*, 209-211), the corresponding phenstatin prodrug **3d** was synthesized (**3b**→**3d**, Scheme 3, Figure 5). Both were used in the previous

phosphorylation techniques (Pettit, et al., Antineoplastic Agents 322., *Anticancer Drug Design* 1995, *supra*; and Pettit, et al., Antineoplastic Agents 320. Synthesis of a Practical Pancratistatin Prodrug, *Anticancer Drug Design* 1995, *10*, 243-250) for such syntheses, based on pentavalent and trivalent phosphorous precursors. However, they proved to be substantially less effective than the dibenzylphosphite approach (Silverberg, et al., A Simple, Rapid and Efficient Protocol for the Selective Phosphorylation of Phenols with Dibenzyl Phosphite, *Tetrahedron Lett.* 1996, *37*, 771-774). The prodrug was synthesized in 3 steps from phenstatin *via* phosphorylation of phenol **3b** employing dibenzylphosphite (under basic conditions in 1:1 acetonitrile - DMF) (*Id.*), followed by cleavage of the benzyl groups **3c** *via* catalytic hydrogenolysis (Pettit, et al., Antineoplastic Agents 320., *Anticancer Drug Design* 1995, *supra*) and reaction of the phosphoric acid product with sodium methoxide in methanol to afford sodium phosphate prodrug **3d** in 56% overall yield.

Accordingly, the prime object of the present invention is to provide an economically viable method for the synthesis of phenstatin and prodrugs thereof which are water soluble and can be used in the preparation of useful antineoplastic preparations.

These and still further objects as shall hereinafter appear are readily fulfilled by the present invention in a remarkably unexpected manner as will be readily discerned from the following detailed description of an exemplary embodiment thereof especially when read in conjunction with the drawings attached hereto.

FIG. 1 is a display of the graphic structures of compounds 1-8 and selected derivatives thereof (as identified in the specification);

FIG. 2 is a computer generated perspective drawing of phenastatin (**3b**);

FIG. 3 is a flow diagram showing the oxidation of Combretastatin A-4 silyl ether (**1c**) to phenstatin silyl ether (**3a**) (herein "Scheme 1");

FIG 4 is a flow diagram of the sequences herein referred to as "Scheme 2"; and

FIG. 5 is a flow diagram showing the conversion of phenstatin (**3b**) to phenstatin prodrug (**3d**) (as identified as "Scheme 3" in the specification).

Compounds **3b**, **3d**, **1d**, and **4a-f** were comparatively evaluated in the NCI 60 cell-line human tumor screen (Boyd, Status of the NCI preclinical antitumor drug discovery screen. Implications for selection of new agents for clinical trial, CANCER: *Principles and Practices of Oncology Updates*, DeVita, et al. (Eds.), Lippincott: Philadelphia, 1989; Vol. 10, No. 3, pp. 1-12; Boyd, The Future of New Drug Development. Section I. Introduction to Cancer Therapy, *Current Therapy in Oncology*, Niederhuber, (Ed.), B.C. Decker, Inc.: Philadelphia, 1993; pp. 11-22; Boyd, et al., Data display and analysis strategies from NCI disease-oriented *in vitro* antitumor drug screen. In *Cytotoxic Anticancer Drugs: Models and Concepts for Drug Discovery and Development*, Valeriote, et al. (Eds.), Kluwer Academic Publishers: Amsterdam, 1992; pp. 11-34; Stinson, et al., Morphological and Immunocytochemical Characteristics of Human Tumor Cell Lines for Use in a Disease-Oriented Anticancer Drug Screen, *Anticancer Res.* 1992, *12*,-1035-1054; Boyd, et al., Some Practical Considerations and Applications of the NCI *in vitro* Drug Discovery Screen, *Drug Dev. Res.* 1995, *34*, 91-109). As illustrated in Table 2, below, the water soluble phenstatin phosphate prodrug **3d** and the parent phenstatin **3b** were essentially indistinguishable in potency (e.g., mean panel GI_{50} values) and differential cytotoxicity profiles (e.g., Compare correlation coefficients). Moreover, **3d** and **3b** were quite similar to the combretastatin A-4 phosphate prodrug **1d** both in terms of potency and differential cytotoxicity. In contrast, the related benzophenones **4a-f** were generally much less potent than **3b**, **3d** or **1d**, although their differential cytotoxicity profiles (and therefore their presumed mechanism of action (Boyd, et al., 1995, *supra*)) did not differ remarkably from

the lead compound. Interestingly, the acetate derivative **3e** of phenstatin exhibited significant human cancer cell line inhibitory activity: e.g., CNS U251 GI_{50} 0.0039 μg/ml, pharynx FADU GI_{50} 0.0055 μg/ml and prostate DU-145 GI_{50} 0.0029 μg/ml.

Table 2. Comparative Evaluations of Phenstatin **3b**, Phenstatin Prodrug **3d**, Combretastatin A-4 Prodrug **1d** and Other Related Benzophenones **4a-f** in the NCI 60 Cell-Line Human Tumor Screen[a]

Compound	Mean Panel GI_{50} (± S.D)[b] .....[x 10^{-8} M]	COMPARE Correlation Coefficient[b]
3b	6.01 (3.76)	1.00
3d	7.33 (4.54)	0.94
1d	1.28 (0.44)	0.81
4a	270. (21.4)	0.74
4b	348. (45.4)	0.79
4c	511. (52.5)	0.74
4d	54.3 (4.94)	0.80
4e	512. (69.8)	0.73
4f	494. (99.0)	0.75

[a]Compounds were tested in triplicate in the NCI screen as described (Boyd, 1993, *supra*; Boyd, et al., 1995, *supra*; Weinstein, et al., An Information-Intensive Approach to the Molecular Pharmacology of Cancer, *Science* 1997, *275*,-343-349) in each of three concentration ranges (HITEST (Boyd, et al., 1995, *supra*) 10^{-5}, 10^{-6}, and 10^{-7}M) using five, 10-fold dilutions within each range. [b]Descriptions and methods of calculation are described elsewhere (*Id.*). COMPARE analyses were performed using the mean graph profiles (*Id.*) of phenstatin **3b** as the seed.

Because of the known potent interactions of combretastatin A-4 **1b** (Pettit, et al., Antineoplastic Agents 291., *J. Med. Chem.* 1995, *supra*) and ketone **3f** (Cushman, et al., Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents, *J. Med. Chem.* 1992, *35*, 2293-2306) with the colchicine site

of tubulin and because of COMPARE studies, such as those shown in Table 2, which suggested similar mechanisms of action, phenstatin **3b**, its prodrug **3d**, and compounds **4a-4f** were evaluated with others for potential inhibitory effects on tubulin polymerization and on the binding of colchicine to tubulin (Table 4, below).

In addition, comparative Murine P388 Lymphocytic Leukemia Cell Line and Human Cancer cell growth inhibitor for phenstatin **3b** vs the phenstatin prodrug **3d** and the combretastatin A4 prodrug **1d** were conducted and are reported in Table 3, below.

Table 3. Comparative Murine P388 Lymphocytic Leukemia Cell Line and Human Cancer Cell Growth Inhibition for Phenstatin **3b** *vs.* the Phenstatin Prodrug **3d** and the Combretastatin A-4 Prodrug **1d**.

Cell Type	Cell Line GI_{50} μg/ml	Phenstatin **3b**	Phenstatin Prodrug **3d** GI_{50} μg/ml	Combretastatin A-4 Prodrug **1d**
Leukemia	P388	0.0033	<0.001	0.0004
Ovarian	OVCAR-3	0.0023	0.0025	0.023
CNS	SF-295	0.052	0.012	0.036
Renal	A498	0.38	0.05	0.041
Lung-NSC	NCI-H460	0.0057	0.035	0.029
Colon	KM20L2	0.04	0.27	0.34
Melanoma	SK-MEL-5	0.0038	0.0047	0.041

Simultaneous studies were performed for comparison of the new compounds with combretastatin A-4 **1b**, its prodrug **1d**, and the related compounds **3f** (Cushman, et al., 1992, *supra*), **1e** (Cushman, et al., Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential

Anticancer Agents That Inhibit Tubulin Polymerization, *J. Med. Chem.* 1991, *34*, 2579-2588), **7a** (Getahun, et al., Synthesis of Alkoxy-Substituted Diaryl Compounds and Correlation of Ring Separation with Inhibition of Tubulin Polymerization: Differential Enhancement of Inhibitory Effects under Suboptimal Polymerization Reaction Conditions, *J. Med. Chem.* 1992, *35*, 1058-1067), **7b** (Cushman, et al., 1992, *supra*), **8a** (Getahun, et al., 1992, *supra*), and **8b** (Cushman, et al., 1991, *supra*).

Table 4. Inhibition of tubulin polymerization and colchicine binding by phenstatin, combretastatin A-4, and related compounds.[a]

Compound	Inhibition of tubulin polymerization IC_{50}, $\mu M \pm SD^b$	Inhibition of colchicine binding % inhibition
1b	1.2 ± 0.1	97
1d	> 80	4
1e	1.3 ± 0.2	97
3b	1.0 ± 0.2	86
3d	21 ± 3	37
3e	3.5 ± 0.5	71
4a	15 ± 2	0
4b	11 ± 2	7
4c	15 ± 2	1
4d	3.6 ± 0.8	58
4f	39 ± 7	0
7a	3.3 ± 0.5	65
7b	4.4 ± 0.2	56
8a	2.1 ± 0.3	77
8b	3.2 ± 0.3	54

[a]Reaction conditions described in detail in the Experimental Section.
[b]SD, standard deviation.

Consistent with the relative antiproliferative activities summarized in Table 4, phenstatin **3b** was equivalent to combretastatin A-4 **1b** in its interactions with tubulin in the assays used here. Confirming unpublished observations with other bulky substituents on the B ring hydroxyl, the combretastatin A-4 prodrug **1d** was totally inactive against purified tubulin, but,

unexpectedly, the phenstatin prodrug **3d** had weak activity in inhibiting assembly and moderate activity as an inhibitor of colchicine binding. In the latter assay the prodrug was about 40% as active as phenstatin.

Previous structure-activity studies (Cushman, et al., *J. Med. Chem.* 1992, *supra*; Getahun, et al., *J. Med. Chem.* 1992, *supra*; Cushman, et al., *J. Med. Chem.* 1991, *supra*) had demonstrated maximal activity with tubulin with a 2-carbon bridge in the Z-stilbene configuration, whether or not the B ring hydroxyl was present, and the data of Table 4 reiterate this conclusion. The tetramethoxy ketone **3f** has shown increased activity relative to the analogous diphenylmethane **7b** (Cushman, et al., *J. Med. Chem.* 1992, *supra*), but the recovery of activity versus the Z-stilbene **1e** was incomplete. In contrast, in the presence of the B ring hydroxyl, the reduced activity of the diphenylmethane **7a** was completely recovered in the ketone phenstatin **3b**.

In terms of the effect of the B ring hydroxyl on interactions with tubulin, no significant difference was observed between the Z-stilbenes **1b** and **1e**, slight differences between the diphenylmethanes **7a** and **7b** and the dibenzyls **8a** and **8b**, and a small difference between the ketones **3b** and **3f**. When a difference was observed, it was always the phenol that had the greater activity.

Manipulation of molecular models demonstrated that it was possible to closely superimpose the two phenyl rings in the ketones with those in the Z-stilbenes, but this was also possible with the bibenzyls. This may indicate that tubulin specifically recognizes the sp^2 hybridization introduced with the carbonyl and stilbene bridges or the ring conjugation, analogous to that in colchicine, that these two bridges permit. The greater activity of **3b** relative to **3f** may also indicate that the B ring phenol group imposes steric constraints on the relative positions of the two phenyl rings.

The series of substituent modifications represented by compounds **4a-4f** failed to yield a derivative superior to **3b** as an inhibitor of either cell growth

or tubulin polymerization. Compound **4a**, which is closely analogous to the A, B, and E rings of podophyllotoxin **5**, had only weak activity against tubulin, and this was comparable to that obtained with **4c**. Compound **4c** has vicinal methoxy groups, with the hydroxyl group of **3b** replaced by a methoxyl group, instead of the methylenedioxy bridge of **4a**. The best activity in this group of compounds was observed with di-*meta* methoxy groups in ring B **4d**, but this compound was 3-4-fold less potent than phenstatin in inhibiting tubulin polymerization. There was a further 3-fold drop in activity with di-*meta* methyl groups in ring B **4b**, and compounds with halogens at these positions **4e** and **4f** were almost inert with tubulin. In the presence of a *para* methoxy group, the bulkiness of the substituent and/or the presence of an oxygen in the *meta* position of ring B seems to be critical for optimum inhibitory activity against tubulin in the ketone derivatives. A hydrogen **3f** leads to greater inhibition than a methoxy or phosphate group **4c, 3d** but is less effective than a hydroxyl **3b**.

Based on the ring substituent structural modifications of phenstatin described herein, both the sp^2 hybridization of the carbonyl carbon that preserves the relative *cis*-relationship of the aromatic rings and the specific 3-hydroxy-4-methoxy-substitution appear necessary for strong cancer cell growth inhibition. Further development of phenstatin and its prodrug are in progress.

General Experimental Procedures. Ether (anhydrous diethyl ether), tetrahydrofuran (distilled from sodium and benzophenone), thionyl chloride and morpholine were distilled prior to use. Reagents were obtained from Sigma-Aldrich Co. Solvent extracts of aqueous solutions were dried over anhydrous magnesium sulfate unless otherwise noted. Flash column chromatography was performed using silica gel (230-400 mesh) and hexane-ethyl acetate as eluant. ANALTECH silica gel GHLF plates were used for TLC. All compounds were visible with fluorescent short-wave light (254 nm).

Melting points were recorded employing an ELECTROTHERMAL 9100 apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded by means of a VARIAN VXR-300 instrument and referenced to chloroform. ^{31}P-NMR was obtained from a VARIAN VXR-500 and referenced to an external standard (85% aqueous H_3PO_4). Mass spectral data were recorded using a VARIAN MAT 312 instrument (EIMS), and IR spectra were determined with a MATTONSON INSTRUMENTS 2020 GALAXY SERIES FTIR instrument. X-ray crystal structure data collection was performed on an ENRAF-NONIUS CAD4 diffractometer.

Jacobsen Oxidation of Combretastatin A-4 **1b**

To a mixture of 4-phenylpyridine-N-oxide (0.127g, 0.74 mmol) and (R,R)-(-)-[N,N^1-bis(3,5-di-*tert*-butylsalicylidene)-1.2-cyclohexanediaminoat(2-)]manganese(III) chloride (Palucki, et al., Highly Enantioselective, Low-Temperature Epoxidation of Styrene, *J. Am. Chem. Soc.* 1994, *116*, 9333-9334) (0.024g, 0.037 mmol) in a 50 mL flask was added a solution of 3'[(*tert*-butyldimethylsilyl)oxy]combretastatin A-4 (**1c**, 0.80g, 1.86 mmol) in 3 Ml of ethyl-acetate (or methylene chloride). The mixture was stirred at 4°C for 20 minutes followed by addition of an aqueous 0.50 M NaOCl solution (6.32 mL, 3.16 mmol) *via* syringe. The temperature remained at 4°C, and reaction progress was monitored by TLC (4:1 hexane-EtOAc). After 5 h, the reaction mixture was warmed to room temperature, and the aqueous phase removed. The organic layer was washed successively with water (2 x 10 mL) and brine (10 mL) and dried over anhydrous Na_2SO_4. Filtration and solvent removal *in vacuo* gave a brown residue that was subjected to flash column chromatography (4:1 hexane-ethyl acetate, 1" x 12" column). The silyl ether protected benzophenone **3a** (Rf = 0.27) was collected as a yellow oil. A second flash column chromatographic separation was necessary for final purification. Consistent 10% yields were obtained based on conversion of combretastatin A-4 silyl ether to phenstatin (**3b**, see below).

Phenstatin 3b.

Silyl ether **3a** (4.90 g, 11.3 mmol) was dissolved in dry tetrahydrofuran (100 mL), and 1.0 M tetrabutylammonium fluoride (11.3 mL, 11.3 mmol) was added (slowly via syringe) while rapidly stirring under Ar (Pietikäinen, *Tetrahedron Lett.* 1995, *supra*). After 15 min, ice (20 g) and ether (100 mL) were added successively. The ethereal layer was washed with water (3 x 100 mL), dried, filtered, and solvent removed under reduced pressure to yield a yellow solid. Flash chromatography (eluant: 3:2 hexane-ethyl acetate, Rf=0.17) was performed and afforded an off-white solid that recrystallized from hexane as colorless granules: EIMS *m/z* (peak height) 318 (M^+, 100%), 303 (14%), 195 (30%), 151 (36%). IR (Nujol, cm^{-1}) 1633 (C=O), 1604 (aromatic C=C); ^{1}H-NMR ($CDCl_3$) δH 7.406 (1H, dd, $J_{6/2}$ 2.1 Hz, $J_{6/5}$ 9.4 Hz, H-6), 7.362 (1H, d, $J_{2/6}$ 2.1 Hz, H-2), 7.011 (2H, s, H-2', 6'), 6.906 (1H, d, $J_{5/6}$ 8.4 Hz, H-5), 5.662 (1H, s, -OH), 3.969 (3H, s, 4'-OCH_3), 3.913 (3H, s, 4-OCH_3), 3.860 (6H, s, 3', 5'-OCH_3); ^{13}C-NMR (75.5 MHZ, $CDCl_3$, assignments deduced with assistance from an APT spectrum) δC 193.3 (C=O), 152.8 (C-3',5'), 150.2 (C-4), 145.3 (C-3), 141.7 (C-4'), 133.2 (C-1'), 131.1 (C-1), 123.6 (C-6), 116.2 (C-2), 110.0 (C-5), 107.5 (C-2',6'), 60.9 (4-OCH_3), 56.3 (3',5'-OCH_3), 56.1 (4'-OCH_3).

Phenstatin acetate 3e.

Acetic anhydride (74 μL, 0.79 mmol) was added to a solution of phenstatin (**3b**, 0.2 g, 0.63 mmol), DMAP (7.8 mg, 0.063 mmol) and triethylamine (0.13 mL, 0.94 mmol) in dry methylene chloride (1.5 mL, under argon at room temperature). The yellow solution was stirred for 30 min (reaction was complete by TLC). Methanol (5 Ml) was added and the reaction mixture concentrated to a solid that was washed with diethyl ether (3 x 10 mL) and partitioned between ethyl acetate and cold Ln hydrochloric acid. The organic layer was washed with 10% aqueous sodium bicarbonate and dried. Solvent removed, *in vacuo*, gave an off-white solid that was recrystallized from ethyl acetate-hexane to give colorless snowflakes of analytical purity: EIMS *m/z*

(peak height) 360 M^+ , 60%), 318 (100%), 303 (10%), 195 (20%), 151 (30%), 91 (32%), 44 (25%). ^{1}H-NMR ($CDCl_3$) δ H 7.740 (1H, dd, $J_{6/2}$ 2.1 Hz, $J_{6/5}$ 8.1 Hz, H-6). 7.556 (1H, d, $J_{2/6}$ 2.1 Hz, H-2), 7.026 (1H, d, $J_{5/6}$ 8.0 Hz, H-5), 7.013 (2H, s, H-2', 6'), 3.914 (3H, s, 4-OCH_3), 3.907 (3H, s, 4'-OCH_3), 3.863 (6H, s, 3', 5'-OCH_3), 2.315 (3H, s, -$OCOCH_3$).

X-Ray Crystal Structure Determination of Phenstatin 3b.

Colorless crystals of ketone **3b** were grown as clusters of thick plates from an ether solution. Cleavage of a single crystal fragment from one of the clusters provided a usable specimen (0.54 x 0.32 x 0.28 mm), which was mounted on the end of a glass capillary tube. Data collection was performed at 25 ± 1°C. Crystal data: $C_{17}H_{18}O_6$, monoclinic space group $P2_1/c$ with a = 12.608(2), b = 14.858(2), c = 8.738(3)Å, β=104.69(2)°, V = 1583.3(12)Å^3, λ(CuKα) = 1.54178 Å, ρ_0 = 1.299 g cm^{-3}, ρ_c = 1.335 g cm^{-3} for Z=4 and F.W. = 318.31, F(000)=672. The structure determined for **3b** converged to the standard crystallographic residual of R_1 = 0.0462 for 2453 reflections in which Fo>4.0 sigma(Fo) and 0.0480 for all 2610 reflections. The corresponding wR2 Sheldrick values, based on Fo^2, were 0.1454 and 0.1475, respectively. The Goodness of Fit for all data was 1.042. Final bond distances and angles were all within acceptable limits.

3-(*tert*-butyldimethylsilyl)oxy-4-methoxybenzoic acid.

To a solution of 3-[*tert*-butyldimethylsilyl)oxy]-4-methoxy-benzaldehyde[2] (35.0 g, 131 mmol) in acetone (400 mL) was added (stirring) a warm solution (40°C) of potassium permanganate (35.3 g, 223 mmol) in water (50 mL)-acetone (250 mL) over 30 min. The reaction mixture was stirred for 45 min (complete by TLC analysis). The suspension was cooled and filtered through a pad of celite, and the filtrate concentrated. The residue was dissolved in ethyl acetate (1000 mL) and transferred to a separatory funnel containing 1 N HCl (800 mL). The organic layer was extracted with water (5 x 1000 mL), brine (500 mL), dried (sodium sulfate), and solvent removed *in vacuo* to give

a crystalline powder. Recrystallization from hexane afforded the benzoic acid as colorless needles (31.0 g, 84% yield): mp=163.5-164.5°C; EIMS *m/z* (peak height) 282 (M^+), 267 (2%), 225 (92%), 210 (100%), 195 (20%).; IR (Nujol, cm^{-1}) 3100 (broad O-H stretch), 1682 (C=O), 1681 (aromatic C=C); ^{1}H-NMR ($CDCl_3$) δH 7.732 (1H, dd, $J_{6/2}$ 2.2 Hz, $J_{6/5}$ 8.4 Hz, H-6), 7.569 (1H, d, $J_{2/6}$ 1.2 Hz, H-2), 6.894 (1H, d, $J_{5/6}$ 8.5 Hz, H-5), 3.882 (3H, s, -OCH_3), 1.009 (9H, s, -Ot-Bu), 0.174 (6H, s, -$Si(CH_2)_{2-}$); ^{13}C-NMR (75.5 MHZ, $CDCl_3$, assignments assisted by an APT spectrum) δC 172.9 (C=O), 156.6 (C-4), 145.4 (C-3), 125.7 (C-2), 123.0 (C-6), 122.4 (C-1), 111.5 (C-5), 55.8 (-OCH_3), 25.9 (-CH_3), -4.5 (-$Si(CH_2)_{2-}$), -4.5 (-Si-C-).

General Procedure for Synthesis of Morpholine Amides **6a-g**.

To a 1.0 M solution of the carboxylic acid chloroform at room temperature was added 2 equivalents of thionyl chloride. The solution was heated to reflux under Ar until TLC (3:1 hexane-ethyl acetate) showed no starting material (approximately 4-8 h). The solution was concentrated to an oil that solidified under high vacuum (0.05 mm Hg), and the crude acid chloride was used in the next step without purification. Two equivalents of morpholine were slowly injected *via* syringe over a 5 min period to a 0.3 M solution of the acid chloride in toluene (under Ar). The reaction mixture was stirred at room temperature until the starting acid chloride was consumed (approx. 1.5-4 h, according to TLC using 3:1 hexane-ethyl acetate). The solution was filtered through a pad of celite to remove morpholine hydrochloride, and the pad washed with toluene. The filtrate was concentrated to an oil that was subjected to flash column chromatography. Concentration of the respective fractions led to the respective amides (Table 1). **6f** and **6g** were synthesized from the acid chloride.

N-[(3-(*tert*butyldimethylsilyl)oxy-4-methoxybenzoyl]morpholine 6a.

A cotton-like solid followed chromatography (eluant: hexane-ethyl acetate 3:2 1:4) and recrystallization from hexane (Table 1). EIMS *m/z* (peak

height) 351 (M^+), 336 (4%), 294 (100%), 278 (26%), 265 (8%), 193 (70%), 165 (16%).

N-(3,5-dimethylbenzoyl)morpholine 6b.

After chromatography (3:2 hexane-ethyl acetate, Rf=0.19), a fluffy-appearing solid was obtained (Table 1).

N-(3,4-dimethoxybenzoyl)morpholine 6c.

Amide **6c** was obtained as a colorless flaky solid (Gardner, et al., The Polyoxyphenols of Western Red Cedar (Thuja Plicata Donn), *Can. J. Chem.* 1960, *38*, 2387-2394) following chromatography (1:2 hexane-ethyl acetate, Rf=0.16).

N-(3,4-methylenedioxybenzoyl)morpholine 6d.

Amide **6d** (Kasztreiner, et al., Synthesis and Pharmacological Investigation of New Alkoxybenzamides II, *Biochem. Pharmacol.* 1962, *11*, 651-657) was isolated as a colorless flaky solid following chromatography (1:1 hexane-ethyl acetate, Rf=0.22).

N-(3,5-dimethoxybenzoyl)morpholine 6e.

After chromatographic (1:1 hexane-ethyl acetate Rf=0.18) purification amide **6e** was collected as a colorless solid. TOF mz/ 251.6 (M+).

N-(3,5-dichlorobenzoyl)morpholine 6f.

The clear oily product crystallized at 0°C: EIMS *m/z* (peak height) 259 (M^+), 261 (M+2), 263 (M+4), 258 (M^+-H), 175 (62%), 173 (96%), 86 (76%), 56 (100%).

N-(3,5-difluorobenzoyl)morpholine 6g.

Amide **6g** (Rf=0.21 in 3:2 hexane-ethyl acetate) gave EIMS *m/z* (peak height) 227 (M^+), 141 (100%), 113 (50%), 86 (36%), 56 (66%).

General Procedures for Synthesis of Benzophenones **3a, 4a-f**

A flame-dried flask containing a 0.1 M solution of 3,4,5-trimethoxybromobenzene (1.1 eq in dry tetrahydrofuran) was cooled to -78°C, evacuated to 1 Torr and refilled with Ar for 10 cycles. To this solution was slowly added *tert*-butyllithium (2.2 eq), and the reaction mixture was stirred for

15 min. A second dry flask containing a 0.1 M solution of the morpholine amide (1.0 eq) in tetrahydrofuran was cooled to -78°C, evacuated, and refilled with Ar. The phenyllithium reagent was transferred *via* cannula to the amide solution. The reaction mixture was stirred at -65 to -78°C for 4 h, followed by a slow warming to room temperature. Progress of the reaction was monitored by TLC (hexane-ethyl acetate). The reaction was stopped by adding 6 eq of isopropyl alcohol and stirring for 1 h. Water was added, and the mixture was extracted with ether (3x). The ethereal extracts were combined, washed with water, dried, and solvent removed to give an oil that was subjected to flash chromatography. Collection and concentration of the required fractions afforded the benzophenone (Table 1).

3-(*tert*-butyldimethylsilyl)oxy- 3',4,4',5'-tetramethoxybenzophenone 3a.

Using amide **6a** (0.20 g, 0.57 mmol), 3,4,5-trimethoxybromobenzene (0.169 g, 0.683 mmol), and t-BuLi (0.74 mL, 1.25 mmol) the above scheme led to crude phenstatin silyl ether **3a** (0.199 g, Rf=0.29 in 4:1 hexane-ethyl acetate). Purification and recrystallization from hexane afforded large glass-like prisms (Table 1); EIMS *m/z* (peak height) 432 (M^+), 417 (2%), 402 (1%), 375 (100%), 360 (58%), 345 (4%), 193 (26%).

3,4,5-trimethoxy-3',4'-methylenedioxybenzophenone 4a.

Flash column chromatography (4:1 hexane-ethyl acetate Rf=0.20) and recrystallization from methanol afforded small colorless needles (Pettit, et al., *J. Med. Pharm. Chem.* 1962, *supra*).

3,4,5-trimethoxy-3',5'-dimethylbenzophenone 4b.

Chromatography (9:1 hexane-ethyl acetate Rf=0.19) and recrystallization from hexane yielded off-white needles: EIMS *m/z* (peak height) 300 (M^+, 100%), 285 (14%), 270 (4%), 375 (100%), 195 (40%).

3,3',4,4',5-pentamethoxybenzophenone 4c.

Purification by column chromatography (4:1 hexane-ethyl acetate Rf=0.10) and recrystallization from toluene-hexane gave an off-white powder:

EIMS *m/z* (peak height) 332 (M^+, 100%), 317 (6%), 301 (8%), 195 (16%), 165 (22%).

3,3',4,5,5'-pentamethoxybenzophenone 4d.

After recrystallization from hexane, the off-white powder did not require chromatographic purification: EIMS *m/z* (peak height) 332 (M^+, 100%), 317 (6%), 301 (8%), 195 (16%), 165 (22%).

3,5-dichloro-3',4',5'-trimethoxybenzophenone 4e.

Chromatographic (9:1 hexane-ethyl acetate, Rf=0.28) separation and recrystallization from hexane afforded clear diamond-shaped crystals: EIMS *m/z* (peak height) 340 (M^+, 100%), 342 (M+2), 344 (M+4), 325 (26%), 310 (4%), 195 (46%).

3,5-difluoro-3',4',5'-trimethoxybenzophenone 4f.

Following chromatography (9:1 hexane-ethyl acetate, Rf=0.21) and recrystallization from hexane, the ketone was obtained as colorless needles: EIMS *m/z* (peak height) 318 (M^+, 100%), 293 (20%), 278 (4%), 195 (22%), 141 (36%), 113 (16%).

3-O-Dibenzylphosphoryl-phenstatin 3c.

To a dry flask equipped with septum, magnetic stirrer, thermometer and Ar inlet containing dry acetonitrile (50 mL) and DMF (50 mL) was added phenstatin (**3b**, 2.0 g, 6.28 mmol). Upon cooling to -10°C, bromotrichloromethane (3.10 mL, 31.4 mmol) was added followed by triethylamine (1.84 mL, 13.2 mmol) and dimethylaminopyridine (77 mg, 0.63 mmol). After one minute, dropwise addition of dibenzylphosphite was begun while maintaining a temperature of -7 to -10°C. The resultant yellow solution was monitored by TLC (1:1 hexane-ethyl acetate, Rf=0.19) until complete. At that time (1.5 h), 0.5 M aqueous KH_2PO_4 (50 mL) was added followed by extraction with ethyl acetate (3 x 100 mL). The organic extract was washed successively with water (200 mL), brine (150 mL), and dried (sodium sulfate). The solution was filtered and concentrated *in vacuo* to a milky oil. Flash

column chromatography (eluant: 1:1 hexane-ethyl acetate) was performed to give benzyl phosphate **3c** as a clear oil (2.60 g, 72% yield): EIMS *m/z* (peak height) (578, M^+), (486, 4%), (91, 100%). ^{1}H-NMR ($CDCl_3$) δH 7.694 (1H, d, $J_{6/5}$ 8.0 Hz, H-6), 7.657 (1H, s, H-2), 7.319 (10H, s, Ar-H), 7.025 (2H, s, H-2',6'), 7.000 (1H, d, $J_{5/6}$ 9.0 Hz, H-5), 5.182 (4H, d, J 8.5 Hz-O-CH_2-Ph), 3.880 (9H, s, 3', 5', 4-OCH_3); ^{31}P-NMR ($CDCl_3$, decoupled, 202.35 MHZ) δP -5.22.

Disodium Phenstatin 3-O-phosphate 3d.

The benzyl phosphate (**3c,** 0.68 g, 1.18 mmol) was added to anhydrous ethyl alcohol (denatured, 50 mL) in a dry flask equipped with a magnetic stirrer. After evacuating the vessel and refilling with Ar (two cycles), 5% palladium-on-carbon (0.58 g) was added. The flask was evacuated and refilled with H_2 to 10 psi (3x). The mixture was stirred vigorously for 15 min and filtered through a 1 cm pad of celite. The flask and celite were washed with ethanol and solvent was removed under reduced pressure to afford a foamy tan solid (0.44 g) that was used in the next step.

The deprotected phosphate (0.44 g, 1.10 mmol) was dissolved in anhydrous methanol (15 mL) and sodium methoxide (88 mg, 2.20 mmol) was added at room temperature. The cloudy solution was stirred for 20 hours and the reaction mixture was concentrated to a white solid that was washed with 2-propanol and recrystallized (3x) from water-acetone. The phenstatin prodrug (**3d**, 0.38g, 78% yield) was collected by filtration and found to give: LRFAB m/z 443.02 (M+H+), calculated 443.048; EIMS m/z (peak height) 318 (100%, M+-PO_3Na_2+H), 303 (12%), 195 (20%), 151 (23%); ^{1}H-NMR (D_2O) δH 7.644 (1H, t, $J_{2/6}$ 1.8 Hz, H-2), 7.366 (1H, dd, $J_{6/2}$ 1.8 Hz, $J_{6/5}$ 8.5 Hz, H-6), 7.006 (1H, d, $J_{5/6}$ 8.5 Hz, H-5), 6.933 (2H, s, H-2',6'), 3.844 (3H, s, 4'-OCH_3), 3.748 (6H, s, 3',5'-OCH_3) 3.734 (3H, s, 4-OCH_3); ^{13}C-NMR (D_2O, reference to $CDCl_3$) δC 193.5 (C=O), 151.1; and ^{31}P-NMR (D_2O, decoupled, -202.35 MHZ) δP -1.965. The solubility of phenstatin prodrug **3d** was found to be 30 mg/mL in distilled water at 25°C.

Tubulin assays.

Electrophoretically homogeneous bovine brain tubulin was purified from bovine brain as described previously (Hamel, et al., Separation of active tubulin and microtubule-associated proteins by ultracentrifugation and isolation of a component causing the formation of microtubule bundles, *Biochemistry* 1984, *23*, 4173-4184). Reaction mixtures (0.24 mL during preincubation, 0.25 mL during incubation, with all concentrations referring to the final volume) contained 0.8 M monosodium glutamate (taken from 2.0 M stock solution adjusted to pH 6.6 with Hcl), 1.0 mg/mL (10 μM) tubulin, 4% (v/v) dimethyl sulfoxide, and varying concentrations of drug. Samples were incubated for 15 min at 30°C and chilled on ice. GTP, required for polymerization, was added in 10 μL to a final concentration of 0.4 mM. Samples were transferred to cuvettes held at 0°C by electronic temperature controllers in GILFORD model 250 recording spectrophotometers. Baselines were established, and the reaction was initiated by a jump (approximately 1 min) to 30°C. Extent of assembly after 20 min was the parameter used to determine IC_{50} values. Active compounds were examined in at least three independent experiments, and inactive compounds were generally evaluated twice. In most experiments, four spectrophotometers were used, with two control reaction mixtures.

The binding of [^{3}H]colchicine to tubulin was measured on DEAE-cellulose filters as described elsewhere (Kang, et al., *N*-acetylcolchinol *O*-methyl ether and thiocolchicine, potent analogs of colchicine modified in the C ring: Evaluation of the mechanistic basis for their enhanced biological properties, *J. Biol. Chem.* 1990, *265*, 10255-10259). Reaction mixtures contained 0.1 mg/mL (1.0 μM) tubulin, 5.0 μM [3H]colchicine, and 5.0 μM inhibitor and were incubated for 10 min at 37°C.

Dosing.

The dosage administered will be dependent upon the identity of the neoplastic disease; the type of host involved, including its age, health and

weight; the kind of concurrent treatment, if any; the frequency of treatment and therapeutic ratio.

Illustratively, dosage levels of the administered active ingredients are: intravenous, 0.1 to about 20 mg/kg; intramuscular, 1 to about 50 mg/kg; orally, 5 to about 100 mg/kg; intranasal instillation, 5 to about 100 mg/kg; and aerosol, 5 to about 100 mg/kg of host body weight.

Expressed in terms of concentration, an active ingredient can be present in the compositions of the present invention for localized use about the cutis, intranasally, pharyngolaryngeally, bronchially, intravaginally, rectally, or ocularly in a concentration of from about 0.01 to about 50% w/w of the composition; preferably about 1 to about 20% w/w of the composition; and for parenteral use in a concentration of from about 0.05 to about 50% w/v of the composition and preferably from about 5 to about 20% w/v.

The compositions of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, suppositories, sterile parenteral solutions or suspensions, sterile non-parenteral solutions of suspensions, and oral solutions or suspensions and the like, containing suitable quantities of an active ingredient.

For oral administration either solid or fluid unit dosage forms can be prepared.

Powders are prepared quite simply by comminuting the active ingredient to a suitably fine size and mixing with a similarly comminuted diluent. The diluent can be an edible carbohydrate material such as lactose or starch. Advantageously, a sweetening agent or sugar is present as well as a flavoring oil.

Capsules are produced by preparing a powder mixture as hereinbefore described and filling into formed gelatin sheaths. Advantageously, as an adjuvant to the filling operation, a lubricant such as talc, magnesium stearate,

calcium stearate and the like is added to the powder mixture before the filling operation.

Soft gelatin capsules are prepared by machine encapsulation of a slurry of active ingredients with an acceptable vegetable oil, light liquid petrolatum or other inert oil or triglyceride.

Tablets are made by preparing a powder mixture, granulating or slugging, adding a lubricant and pressing into tablets. The powder mixture is prepared by mixing an active ingredient, suitably comminuted, with a diluent or base such as starch, lactose, kaolin, dicalcium phosphate and the like. The powder mixture can be granulated by wetting with a binder such as corn syrup, gelatin solution, methylcellulose solution or acacia mucilage and forcing through a screen. As an alternative to granulating, the powder mixture can be slugged, i.e., run through the tablet machine and the resulting imperfectly formed tablets broken into pieces (slugs). The slugs can be lubricated to prevent sticking to the tablet-forming dies by means of the addition of stearic acid, a stearic salt, talc or mineral oil. The lubricated mixture is then compressed into tablets.

Advantageously, the tablet can be provided with a protective coating consisting of a sealing coat or enteric coat of shellac, a coating of sugar and methylcellulose and polish coating of carnauba wax.

Fluid unit dosage forms for oral administration such as in syrups, elixirs and suspensions can be prepared wherein each teaspoonful of composition contains a predetermined amount of an active ingredient for administration. The water-soluble forms can be dissolved in an aqueous vehicle together with sugar, flavoring agents and preservatives to form a syrup. An elixir is prepared by using a hydroalcoholic vehicle with suitable sweeteners together with a flavoring agent. Suspensions can be prepared of the insoluble forms with a suitable vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose and the like.

For parenteral administration, fluid unit dosage forms are prepared utilizing an active ingredient and a sterile vehicle, water being preferred. The active ingredient, depending on the form and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the water-soluble active ingredient can be dissolved in water for injection and filter sterilized before filling into a suitable vial or ampule and sealing. Advantageously, adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. Parenteral suspensions are prepared in substantially the same manner except that an active ingredient is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The active ingredient can be sterilized by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

In addition to oral and parenteral administration, the rectal and vaginal routes can be utilized. An active ingredient can be administered by means of a suppository. A vehicle which has a melting point at about body temperature or one that is readily soluble can be utilized. For example, cocoa butter and various polyethylene glycols (Carbowaxes) can serve as the vehicle.

For intranasal instillation, a fluid unit dosage form is prepared utilizing an active ingredient and a suitable pharmaceutical vehicle, preferably P.F. water, a dry powder can be formulated when insufflation is the administration of choice.

For use as aerosols, the active ingredients can be packaged in a pressurized aerosol container together with a gaseous or liquified propellant, for example, dichlorodifluoromethane, carbon dioxide, nitrogen, propane, and the like, with the usual adjuvants such as cosolvents and wetting agents, as may be necessary or desirable.

The term "unit dosage form" as used in the specification and claims refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical diluent, carrier or vehicle. The specifications for the novel unit dosage forms of this invention are dictated by and are directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitation inherent in the art of compounding such an active material for therapeutic use in humans, as disclosed in this specification, these being features of the present invention. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, troches, suppositories, powder packets, wafers, cachets, teaspoonfuls, tablespoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and other forms as herein described.

The active ingredients to be employed as antineoplastic agents can be easily prepared in such unit dosage form with the employment of pharmaceutical materials which themselves are available in the art and can be prepared by established procedures. The following preparations are illustrative of the preparation of the unit dosage forms of the present invention, and not as a limitation thereof. Several dosage forms were prepared embodying the present invention. They are shown in the following examples in which the notation "active ingredient" signifies either phenstatin **3b** and/or phenstatin prodrug **3d**, and/or benzophenones **4a-f** or any other compound described herein.

COMPOSITION "A"

Hard-Gelatin Capsules

One thousand two-piece hard gelatin capsules for oral use, each capsule containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	200 g
Corn Starch	20 g
Talc	20 g
Magnesium stearate	2 g

The active ingredient, finely divided by means of an air micronizer, is added to the other finely powdered ingredients, mixed thoroughly and then encapsulated in the usual manner.

The foregoing capsules are useful for treating a neoplastic disease by the oral administration of one or two capsules one to four times a day.

Using the procedure above, capsules are similarly prepared containing an active ingredient in 50, 250 and 500 mg amounts by substituting 50 g, 250 g and 500 g of an active ingredient for the 200 g used above.

COMPOSITION "B"

Soft Gelatin Capsules

One-piece soft gelatin capsules for oral use, each containing 200 mg of an active ingredient, finely divided by means of an air micronizer, are prepared by first suspending the compound in 0.5 ml of corn oil to render the material capsulatable and then encapsulating in the above manner.

The foregoing capsules are useful for treating a neoplastic disease by the oral administration of one or two capsules one to four times a day.

COMPOSITION "C"

Tablets

One thousand tablets, each containing 200 mg of an active ingredient, are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	200 g
Lactose	300 g
Corn starch	50 g
Magnesium stearate	4 g
Light liquid petrolatum	5 g

The active ingredient, finely divided by means of an air micronizer, is added to the other ingredients and then thoroughly mixed and slugged. The slugs are broken down by forcing them through a Number Sixteen screen. The resulting granules are then compressed into tablets, each tablet containing 200 mg of the active ingredient.

The foregoing tablets are useful for treating a neoplastic disease by the oral administration of one or two tablets one to four times a day.

Using the procedure above, tablets are similarly prepared containing an active ingredient in 250 mg and 100 mg amounts by substituting 250 g and 100 g of an active ingredient for the 200 g used above.

COMPOSITION "D"

Oral Suspension

One liter of an aqueous suspension for oral use, containing in each teaspoonful (5 ml) dose, 50 mg of an active ingredient, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	10 g
Citric acid	2 g
Benzoic acid	1 g
Sucrose	790 g
Tragacanth	5 g
Lemon Oil	2 g
Deionized water, q.s. 1000 ml	

The citric acid, benzoic acid, sucrose, tragacanth and lemon oil are dispersed in sufficient water to make 850 ml of suspension. The active ingredient, finely divided by means of an air micronizer, is stirred into the syrup unit uniformly distributed. Sufficient water is added to make 1000 ml.

The composition so prepared is useful for treating a neoplastic disease at a dose of 1 teaspoonful (15 ml) three times a day.

COMPOSITION "E"

Parenteral Product

A sterile aqueous suspension for parenteral injection, containing 30 mg of an active ingredient in each milliliter for treating a neoplastic disease, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	30 g
POLYSORBATE 80	5 g
Methylparaben	2.5 g
Propylparaben	0.17 g
Water for injection, q.s. 1000 ml.	

All the ingredients, except the active ingredient, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized active ingredient, finely divided by means of an air micronizer, and the final suspension is filled into sterile vials and the vials sealed.

The composition so prepared is useful for treating a neoplastic disease at a dose of 1 milliliter (1ml) three times a day.

COMPOSITION "F"

Suppository, Rectal and Vaginal

One thousand suppositories, each weighing 2.5 g and containing 200 mg of an active ingredient are prepared from the following types and amounts of ingredients:

Active ingredient, micronized	15 g
Propylene glycol	150 g
Polyethylene glycol #4000, q.s.	2,500 g

The active ingredient is finely divided by means of an air micronizer and added to the propylene glycol and the mixture passed through a colloid mill until uniformly dispersed. The polyethylene glycol is melted and the propylene glycol dispersion is added slowly with stirring. The suspension is poured into

unchilled molds at 40°C. The composition is allowed to cool and solidify and then removed from the mold and each suppository foil wrapped.

The foregoing suppositories are inserted rectally or vaginally for treating a neoplastic disease.

COMPOSITION "G"

Intranasal Suspension

One liter of a sterile aqueous suspension for intranasal instillation, containing 20 mg of an active ingredient in each milliliter, is prepared from the following types and amounts of ingredients:

Active ingredient, micronized	15 g
POLYSORBATE 80	5 g
Methylparaben	2.5 g
Propylparaben	0.17 g
Deionized water, q.s. 1000 ml.	

All the ingredients, except the active ingredient, are dissolved in the water and the solution sterilized by filtration. To the sterile solution is added the sterilized active ingredient, finely divided by means of an air micronizer, and the final suspension is aseptically filled into sterile containers.

The composition so prepared is useful for treating a neoplastic disease, by intranasal instillation of 0.2 to 0.5 ml given one to four times per day.

An active ingredient can also be present in the undiluted pure form for use locally about the cutis, intranasally, pharyngolaryngeally, bronchially, or orally.

COMPOSITION "H"

Powder

Five grams of an active ingredient in bulk form is finely divided by means of an air micronizer. The micronized powder is placed in a shaker-type container.

The foregoing composition is useful for treating a neoplastic disease, at localized sites by applying a powder one to four times per day.

COMPOSITION "I"

Oral Powder

One hundred grams of an active ingredient in bulk form is finely divided by means of an air micronizer. The micronized powder is divided into individual doses of 200 mg and packaged.

The foregoing powders are useful for treating a neoplastic disease, by the oral administration of one or two powders suspended in a glass of water, one to four times per day.

COMPOSITION "J"

Insufflation

One hundred grams of an active ingredient in bulk form is finely divided by means of an air micronizer.

The foregoing composition is useful for treating a neoplastic disease, by the inhalation of 300 mg one to four times a day.

From the foregoing, it becomes readily apparent that a new and useful antineoplastic factor and new and useful antineoplastic preparations have been herein described and illustrated which fulfill all of the aforestated objectives in a remarkably unexpected fashion. It is of course understood that such modifications, alterations and adaptations as will readily occur to the artisan confronted with this disclosure are intended within the spirit of the present invention.